

AD _____

GRANT NUMBER DAMD17-96-1-6221

TITLE: Importance of USF in Breast Cancer

PRINCIPAL INVESTIGATOR: Michele Sawadogo, Ph.D.

CONTRACTING ORGANIZATION: The University of Texas
M. D. Anderson Cancer Center
Houston, Texas 77030

REPORT DATE: September 1998

TYPE OF REPORT: Final

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
distribution unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED	
	September 1998	Final (1 Sep 96 - 31 Aug 98)	
4. TITLE AND SUBTITLE Importance of USF in Breast Cancer			5. FUNDING NUMBERS DAMD17-96-1-6221
6. AUTHOR(S) Michele Sawadogo, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas M. D. Anderson cancer Center Houston, Texas 77030			8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, Frederick, Maryland 21702-5012			10. SPONSORING/MONITORING AGENCY REPORT NUMBER
11. SUPPLEMENTARY NOTES 19981231 050			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 USF is a family of transcription factors that can antagonize the activity of the Myc oncoproteins in cellular proliferation and transformation. The abundance and activity of the USF proteins in normal and tumorigenic breast epithelial cells were analyzed. All cell lines tested contained similar USF DNA-binding activity and similar expression levels of the ubiquitous USF1 and USF2 polypeptides. In MCF-10A, a normal breast epithelial cell line, the USF proteins acted as strong transcriptional activators at promoters containing USF-specific binding sites. In contrast, the transcriptional activity of USF was either decreased or absent in several breast cancer cell lines. This partial or complete loss of USF function is likely to contribute to the uncontrolled proliferation and tumorigenicity of breast cancer cells.			
14. SUBJECT TERMS Breast Cancer transcription factors, USF, Myc, tumor suppressors, breast cancer cell lines, loss-of-function mutations			15. NUMBER OF PAGES 18
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

Where copyrighted material is quoted, permission has been obtained to use such material.

Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

ms For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

ms In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

ms In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

ms In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

m. g

9/29/98

PI - Signature

Date

TABLE OF CONTENT

Front cover	page 1
Standard Form	page 2
Foreword	page 3
Table of content	page 4
Introduction	pages 5-6
Body	pages 6-15
Conclusion	page 15
References	pages 16-17
Publications	page 18
List of personnel	page 18

Grant DMAD17-96-1-6221, Role of USF in breast cancer

Michele Sawadogo, Ph.D., Principal Investigator

Progress report: September 1, 1996-August 31, 1998.

INTRODUCTION

The purpose of this grant was to investigate the role of the USF proteins in breast cancer. The major reason to initiate these studies was the demonstrated antagonism between the cellular function of USF and that of the Myc oncoproteins. Myc overexpression, whether due to gene translocation, amplification or increased message stability, is known to play a key role in cancer progression (1, 2). For breast tumors, Myc overexpression is a very strong indicator of an increased risk of relapse, especially in the case of node-negative patients (3, 4).

Myc and USF both belong to the basic-helix-loop-helix-leucine zipper family of transcriptional regulators. They also share a common DNA-binding specificity for sites containing a 5' CACGTG 3' core sequence (5-8) and are both indispensable during embryonic development (9, 10). However, the Myc proteins promote cellular proliferation and their overexpression leads to uncontrolled growth (1). In contrast, studies in our laboratory revealed that the USF proteins have antiproliferative activities (11). We showed that USF overexpression specifically inhibited the cellular transformation of primary embryo fibroblasts mediated by c-Myc and activated Ras. Constitutive expression of certain forms of USF also inhibited the proliferation of several transformed cell lines. Our results therefore suggested that one of the normal functions of USF was to protect the cells against the oncogenic potential of Myc (11).

Given these observations, we proposed in this grant to:

Specific aim #1: Investigate the expression of the various USF isoforms in breast cancer cells from different origins.

Specific aim #2: Determine whether overexpression of USF could inhibit the proliferation of breast cancer cells, which would open a novel opportunity for gene therapy.

Completion of these specific aims required the following tasks:

- 1) Obtain different breast cancer cell lines, expand the original aliquot, and freeze for long term conservation.
- 2) Characterize the forms of USF present in different breast cancer cells, using nuclear extracts and electrophoretic mobility shift assays.
- 3) Optimize transfection methods to be used with different breast cancer cell lines.
- 4) Establish and characterize breast cancer cell lines overexpressing USF.

As detailed below, we have accomplished all of these tasks. Furthermore, we demonstrated the existence of a frequent loss of USF function in breast cancer cells as compared to normal breast epithelial cells. This result has opened entirely new areas of investigations and can eventually lead to the discovery of a new tumor suppressor gene.

RESULTS

A. Tasks accomplished

Task 1: We have cultured, frozen aliquots, and analyzed eight normal and breast cancer cell lines (MCF-10A, HBL100, MCF-7, BT-20, Hs578T, T47D, MDA-MB 231 and MDA-MB-468).

Task 2: We have prepared nuclear extracts from these different cell lines and analyzed the levels of endogenous USF using Western blot analysis and electrophoretic mobility shift assay (EMSA).

Task 3: We have investigated the efficiency of different transfection methods and succeeded in optimizing conditions for seven of these cell lines.

Task 4: Using these optimized conditions, we have carried out both transient and stable transfection assays to analyze the effect of USF overexpression in these different cell lines.

B. Expression of the different USF isoforms in various breast and breast cancer cell lines (specific aim 1)

Two different genes, *Usf1* and *Usf2*, encode all ubiquitously expressed USF proteins (10, 11). Analysis by Western blotting and EMSA did not reveal any significant difference in the absolute or relative abundance of the USF1 and USF2-containing homo- and heterodimers in various normal and breast cancer cell lines. The expression of USF

in breast epithelial cells was furthermore very similar to that previously characterized in other human cell lines such as HeLa and Saos-2 (Fig. 1). Eight different breast cell lines, originating from either tumors or normal tissue, were included in this analysis. From these results, we concluded that, if the USF transcription factors were implicated in breast carcinogenesis, changes in the expression or DNA-binding activity of USF were probably not involved.

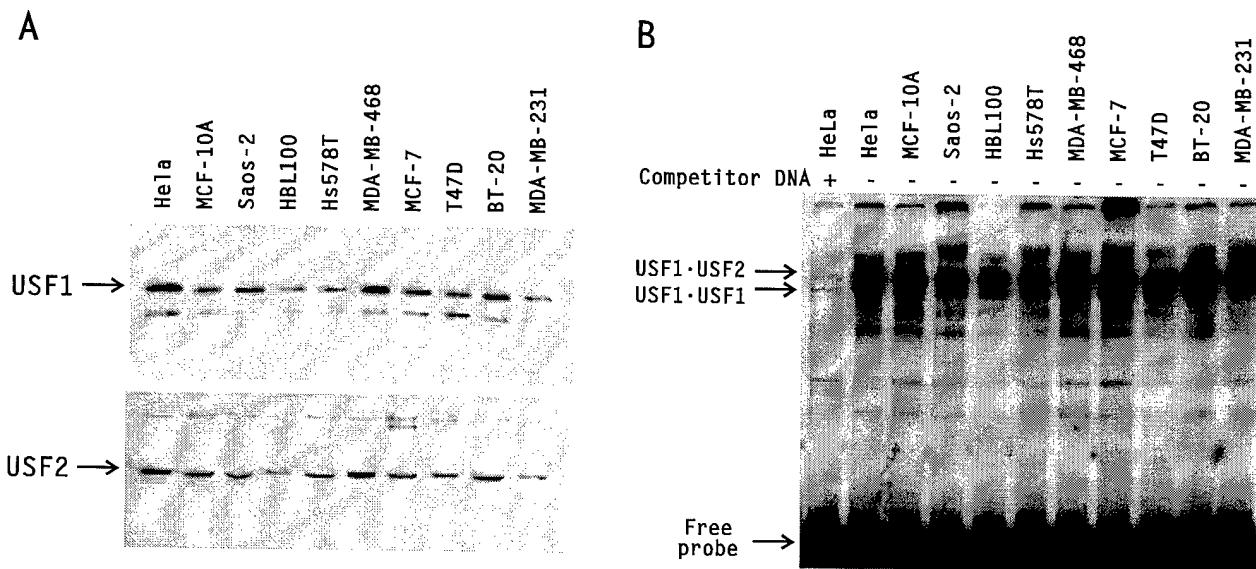


Figure 1: Expression and DNA-binding activity of the USF proteins in eight different breast cell lines. Mini nuclear extracts (13) were prepared for each cell line under identical conditions. HeLa and Saos-2 cells, where the activity of USF is currently best characterized, were included for direct comparison. Extracts were made in each case from 10^6 cells and identical volumes of nuclear extracts were used in each lane.

(A) Western blot analysis. USF1 and USF2 levels were analyzed by probing the same blot successively with USF2- and USF1-specific rabbit peptide antibodies (Santa Cruz Biotechnology). Migration of the 43-kDa USF1 and 44-kDa USF2 polypeptides is indicated at left.

(B) Analysis of endogenous USF DNA-binding activity by EMSA. DNA-binding reactions were assembled using a 150-bp radiolabeled DNA fragment containing a USF-specific binding site. This probe and electrophoresis conditions were chosen to optimize resolution of the different USF dimers (12). Competitor DNA under the form of a 30-bp oligonucleotide containing the USF consensus binding site was included in the reactions as indicated. Migration of the major USF complexes present in all cell lines is indicated at left.

C. Effect of USF overexpression in breast cancer cell lines (specific aim 2)

1) Optimized conditions for transfecting different breast cell lines: We have explored the use of different transfecting agents at various concentrations and with different incubation times in order to optimize our transfection efficiencies in each cell line. Conditions yielding high transfection levels are summarized in Table 1. Transfection efficiencies in BT-20 cells were extremely low under all conditions tested, so this cell line was not used to investigate the effects of exogenous USF expression.

Cell line	Number of cells per well	Transfecting agent	Total DNA	Incubation time
MCF-10A	7×10^5	Lipofectamine (10 μ l)	2 μ g	5 h
Hs578T	2.5×10^5	Lipofectamine (7.5 μ l)	2 μ g	5 h
HBL100	5×10^5	Fugene 6 (3 μ l)	2 μ g	46-50 h
MDA-MB-468	7×10^5	Fugene 6 (6 μ l)	2 μ g	46-50 h
MCF-7	2.5×10^5	Lipofectin (5 μ l)	2.5 μ g	6 h
MDA-MB-231	5×10^5	Fugene 6 (6 μ l)	1 μ g	46-50 h
T47D	7×10^5	Fugene 6 (3 μ l)	2 μ g	46-50 h

Table 1: Conditions yielding efficient transfections in various breast cell lines. Prior to transfection, the indicated number of cells were seeded in 6-well plates. The transfecting agent and total amount of transfected plasmid DNA, as well as the length of the incubation period during which the cells were left in contact with the DNA and transfecting agent, are also indicated. For transient transfection assays, the cells were collected 46-50 hours after transfection and extracts prepared for luciferase assay.

Using these optimized conditions, we compared the transfection efficiency of the different cell lines using the pRSV-Luc reporter plasmid in which luciferase expression is driven by the ubiquitously active Rous sarcoma virus promoter. These experiments revealed that the transfection efficiencies were not too dissimilar, with less than 4 fold difference between the largest and smallest values of luciferase activity (Fig. 2). HBL100 and MDA-MB-468 were the two cell lines that transfected with the lowest efficiency.

EMSA was used to monitor our ability to overexpress USF in the same cell lines. In all cases, high overexpression was observed after transient transfection of expression vectors encoding either USF1, USF2, or the deletion mutant USF2 Δ N (Fig. 3). The latter

construct contains only the DNA-binding domain of USF2 and was useful to determine the expression of reporter genes in the absence of USF transcriptional activity.

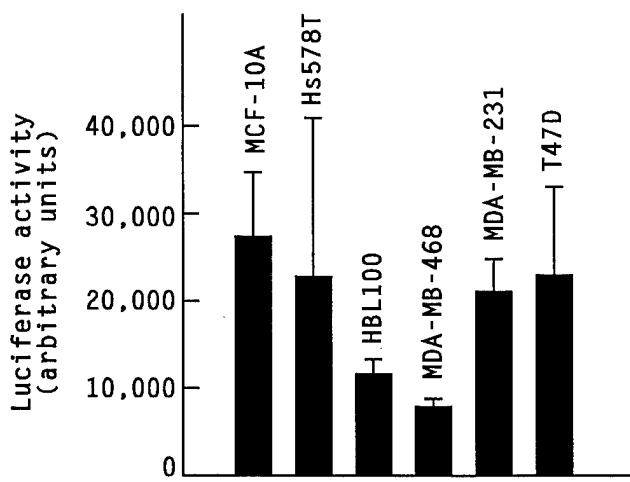


Figure 2: Activity of the RSV-Luc reporter in different breast cell lines. Transfections were carried out as described in Table I. The resulting luciferase activities were monitored using a scintillation counter and are expressed in arbitrary units.

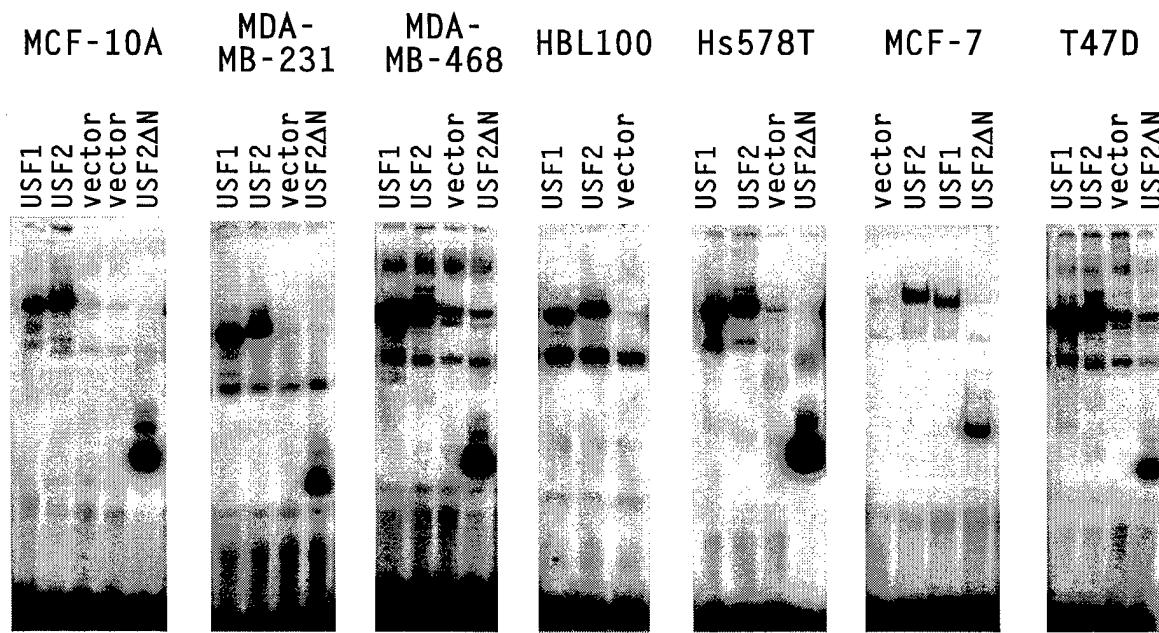


Figure 3: Expression of exogenous USF in different breast cell lines. Cells were transfected as described in Table I with expression vectors encoding either USF1, USF2, or USF2 Δ N, or with the corresponding empty vector, as indicated above each lane. USF overexpression was monitored by EMSA using a radiolabeled 33-bp oligonucleotide containing the USF consensus binding site.

2) Transcriptional activity of USF in normal and breast cancer cell lines: To investigate the transcriptional activity of USF in the different cell lines, we used transient cotransfection assays with the reporter plasmids shown in Fig. 4A. Both pMLLuc and pU3MLLuc contain the adenovirus major late minimum promoter driving transcription of the luciferase gene. The two reporters differ only in the presence or absence of 3 USF-specific binding sites inserted in pU3MLLuc upstream of the TATA box. Our earlier studies had shown that the activity of the pU3MLLuc reporter is strongly activated in HeLa cells by cotransfection with either USF1 or USF2 (14).

a. Transcriptional activity of USF in normal breast epithelial cells: We used MCF-10A, a spontaneously immortalized cell line, to evaluate the transcriptional activity of USF in normal breast epithelial cells (Fig. 4). In these cells, transfection of the pU3MLLuc reporter yielded a luciferase activity 13 fold higher than that observed with pMLLuc, indicating that endogenous proteins activated transcription by binding to the USF sites (Fig. 4B). To determine whether these endogenous proteins were USF itself, we cotransfected expression vectors encoding dominant negative mutants for either the USF or the Myc transcription factors. A-USF and A-Max are constructs in which the basic region of USF1 or Max, respectively, was replaced by an acidic sequence (15, 16). This substitution greatly stabilizes heterodimer formation. For example, at a 3:1 ratio, A-USF essentially abolishes DNA binding by USF in vitro (15). Cotransfection of A-USF significantly reduced the activity of the pU3MLLuc reporter in MCF-10A cells, while cotransfection of A-Max was much less inhibitory. Cotransfection of either A-USF or A-Max had essentially no effect on the activity of the pMLLuc reporter (Fig. 4B). From these results, we concluded that endogenous USF, rather than Myc-related proteins, was primarily responsible for the activity of the pU3MLLuc reporter in MCF-10A cells.

The ability of exogenous USF proteins to function as transcriptional activators in MCF-10A cells was monitored by cotransfected pU3MLLuc and expression vectors for either USF1, USF2, or the USF2 Δ N mutant lacking all transcriptional activation domains. As shown in Fig. 4C, transcription from the pU3MLLuc reporter was increased by cotransfection of USF1 and very strongly increased by cotransfection of USF2, indicating that exogenous USF proteins were also active in MCF-10A cells. In contrast, cotransfection of USF2 Δ N strongly repressed the transcription of pU3MLLuc, probably by preventing the binding of endogenous USF to the promoter DNA (Fig. 4C). From these experiments, we concluded that both endogenous and exogenous USF proteins were active transcription factors in normal breast epithelial cells.

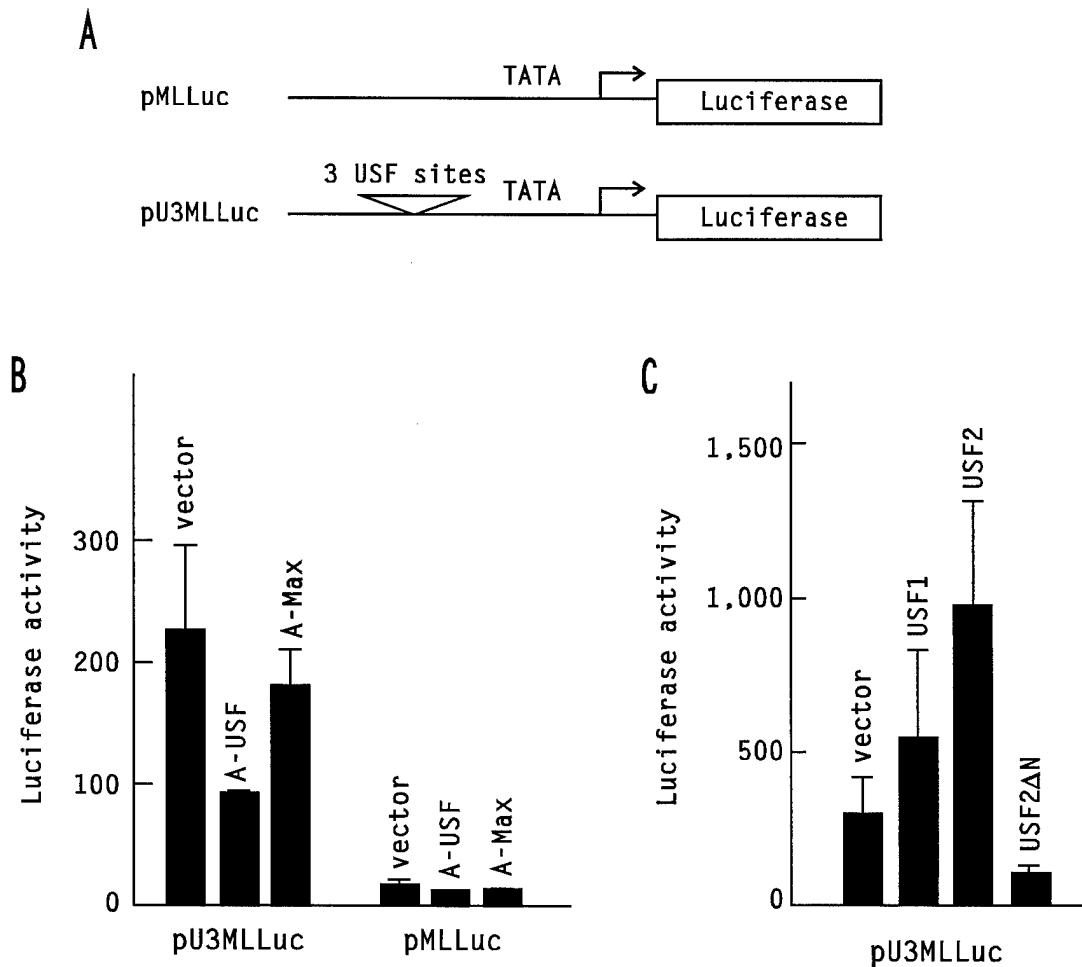


Figure 4: The USF proteins are transcriptionally active in normal breast epithelial cells.
(A) Schematic representation of the reporter plasmids. **(B)** Transcriptional activity of endogenous USF in MCF-10A cells was monitored by transfecting either the pMLLuc or the pU3MLLuc reporter in the presence or absence of dominant negative mutants of the USF or Myc transcription factors, as indicated. **(C)** The transcriptional activity of exogenous USF1, USF2 or USF2 Δ N in MCF-10A cells was monitored by cotransfection with the pU3MLLuc reporter.

b) Evidence for a partial or complete loss of USF transcriptional activity in breast cancer cell lines: In contrast to the pRSV-Luc reporter, which yielded comparable luciferase activities in all cell lines (Fig. 2), the USF-dependent pU3MLLuc reporter was very poorly transcribed in all six tumorigenic breast cell lines tested as compared to MCF-10A cells (Fig. 5). This was surprising since USF was present at similar levels in all these cell lines (Fig. 1). Given the evidence for a strong transcriptional activity of endogenous USF in MCF-10A (Fig. 4B), these results suggested that endogenous USF exhibited a decreased transcriptional activity in cancer cells as compared to normal cells.

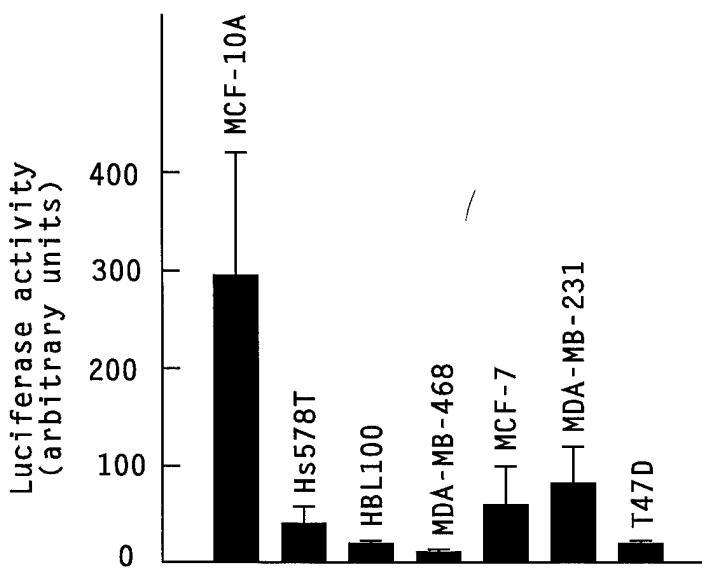


Figure 5: Activity of endogenous transcription factors at the pU3MLLuc reporter in different cell lines.

The ability of exogenous USF1 and USF2 to activate transcription in different breast cancer cell lines was next analyzed by cotransfection assay using the pU3MLLuc reporter and USF2 Δ N as a negative control (Fig. 6). In three cell lines, Hs578T, MDA-MB-468 and T47D, strong activity was detected for USF1 (13 to 22 fold activation), while USF2 stimulated transcription only 2 to 3 fold over the endogenous level. Given that USF2 was actually more active than USF1 in MCF-10A, this result suggested a deficiency affecting specifically the transcriptional activity of USF2 in these 3 cell lines. Interestingly, USF1 and USF2 were both inactive in MCF-7, MDA-MB-231 and HBL100. This result indicated a loss of USF function in these cell lines very similar to that previously characterized in our laboratory for the Saos-2 osteosarcoma cell line (15). In Saos-2 cells, the inactivity of the USF proteins was linked to the inactivity of the USF-specific region (USR), a transcriptional activation domain that is highly conserved in USF1 and USF2 (15). Together, these observations can be explained if a specialized coactivator, which normally interacts with the USR domain to mediate the transcriptional activity of both USF1 and USF2, is absent in Saos-2 cells as well as in several breast cancer cell lines. If this explanation is correct, the loss of this USF-specific transcriptional coactivator may well play a key role in carcinogenesis. Therefore, cloning and identification of the corresponding gene has now become a major goal in our laboratory.

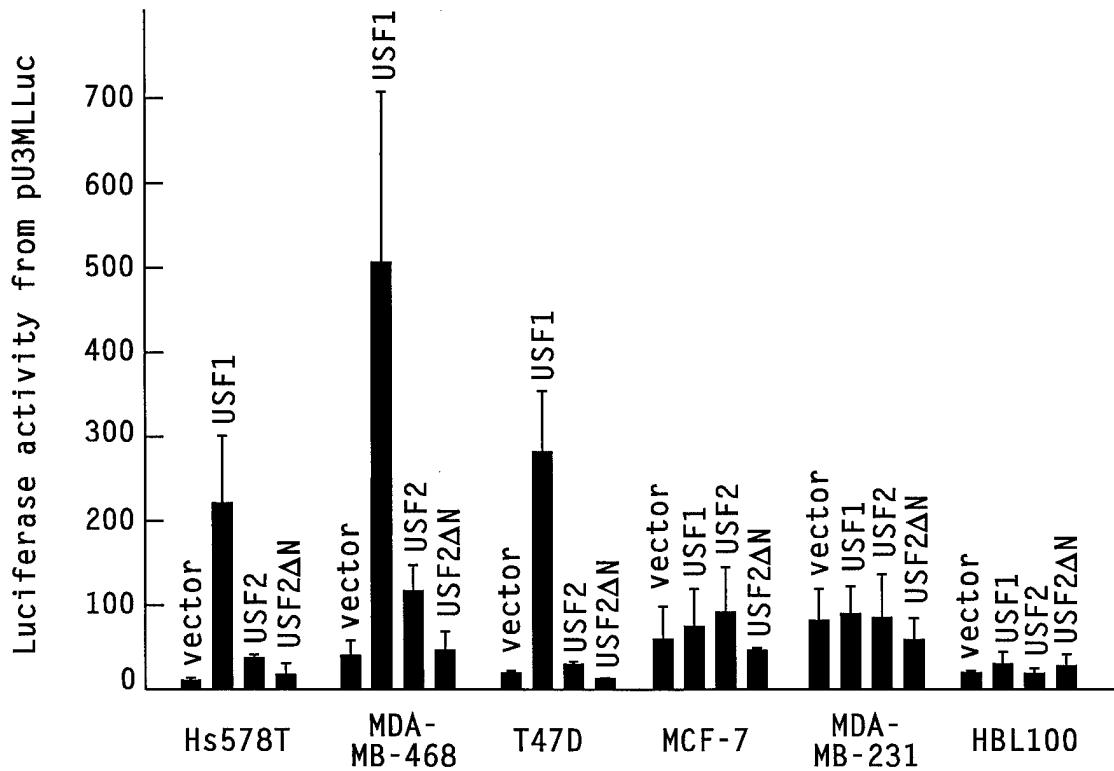


Figure 6: Transcriptional activity of exogenous USF in the different cell lines. The transcriptional activity of exogenous USF proteins in different cell lines was determined by cotransfected pU3MLLuc with the indicated USF expression vectors.

c) Effect of USF overexpression on the proliferation of normal and breast cancer cell lines: In HeLa cells, where the USF proteins are transcriptionally active, overexpression of USF2 was found to drastically inhibit growth (11, 15). Therefore, when we initiated the studies reported here, one of our goals was to determine whether overexpression of USF proteins, and in particular overexpression of USF2, could similarly inhibit the proliferation of breast cancer cells. However, in HeLa cells, growth inhibition by USF2 requires it to be transcriptionally active (11). Given the general loss of USF2 transcriptional activity in breast cancer cells (Fig. 6), it seemed therefore unlikely that USF2 overexpression would be growth inhibitory in these cells. Nevertheless, we carried out a few experiments using colony formation assays to monitor the effect of USF overexpression on proliferation. Overexpression of USF1 or USF2 had essentially no effect on the colony plating ability of MCF-10A, Hs578T, or MCF-7 (Table 2). In Hs578T and MCF-7 cells, the lack of effect of USF2 on proliferation is probably related to its lack of transcriptional activity, as previously found in the case of the Saos-2 cell

line (15). On the other hand, the ability of MCF-10A cells to proliferate in the presence of elevated levels of transcriptionally active USF2 is interesting since it suggests that the growth inhibition by USF may be specific to transformed cells.

Cotransfected plasmids:	MCF-10A colonies		Hs578T colonies	MCF-7 colonies
	Exp. 1	Exp. 2		
pSV2neo	115	179	108	614
pSV2neo + USF1	73	77	187	622
pSV2neo + USF2	80	116	139	583
pSV2neo + USF2ΔN	45	86	183	674

Table 2: Effect of USF overexpression on the proliferation of MCF-10A, Hs578T and MCF-7 cells. Cells were cotransfected with pSV2neo and the indicated USF expression vectors. After 3 weeks of selection in the presence of G418, resistant colonies were stained with crystal violet and counted.

3) Model for the antagonistic roles of USF and Myc in the control of cellular proliferation: Our experiments indicate that a partial or complete loss of USF function is a common feature in cancer cells in general, and in breast cancer cells in particular. Given the antiproliferative properties of the USF proteins in other cell lines and also their ability to antagonize the transforming activity of Myc (11), we like to propose a model in which cellular proliferation is regulated in normal cells by the opposing effects of USF and Myc. In cancer cells, uncontrolled proliferation may be triggered by the overexpression of Myc and/or by the inactivation of USF (Fig. 7). This model accounts for the fact that both Myc overexpression and USF inactivation are common events in cancer cell lines. Whether USF is also inactivated in tumor cells *in vivo* remains however to be determined.

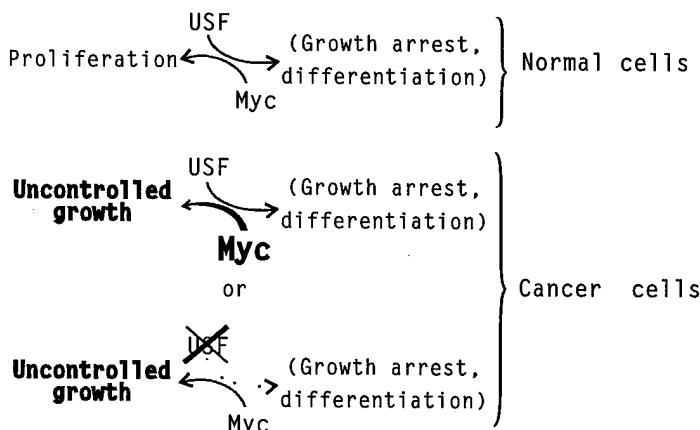


Figure 7: Schematic representation of a model for the antagonism between USF and Myc in the control of cellular proliferation.

CONCLUSIONS

We have analyzed the abundance and activity of the USF transcription factors in normal and tumorigenic breast epithelial cells. Similar USF DNA-binding activities and similar expression levels of the ubiquitous USF1 and USF2 polypeptides were observed in all cell lines tested. In MCF-10A, a normal breast epithelial cell line, strong USF transcriptional activity was demonstrated for both endogenous proteins and exogenously expressed USF1 and USF2. In contrast, breast cancer cell lines exhibited either a normal USF1 activity but greatly diminished USF2 activity or a complete loss of transcriptional activity for both USF1 and USF2. This partial or complete loss of USF function in breast cancer cells is likely to contribute to the uncontrolled proliferation and tumorigenicity of these cells. Further studies will be necessary to characterize the genetic alteration(s) that are responsible for the inactivity of USF in different breast cancer cells and determine whether these alterations involve a novel tumor suppressor gene.

REFERENCES

- 1) Koskinen, P. J. and K. Alitalo (1993). Role of myc amplification and overexpression in cell growth, differentiation and death. Cancer Biol. **4**:3-12.
- 2) Marcu, K.B., S.A. Bossone, and A.J. Patel (1992). Myc function and regulation. Annu. Rev. Biochem. **61**:809-860.
- 3) Berns, E.M.J.J., F.G. Klijn, W.L.J. van Putten, I.L. van Staveren and H. Portengen (1992). C-myc amplification is a better prognostic factor than Her2/neu amplification in primary breast cancer. Cancer Res. **52**:1107-1113.
- 4) Kreipe, H., H. Feist, L. Fisher, J. Felgner, K. Heidorn, L. Mettler and R. Parwaresch (1993) Amplification of c-myc but not c-erbB-2 is associated with high proliferative capacity in breast cancer. Cancer Res. **53**:1956-1961.
- 5) Murre, C., P.S. McCaw and D. Baltimore (1989). A new DNA binding and dimerization motif in immunoglobulin enhancer binding, daughterless, MyoD, and myc proteins. Cell **56**: 777-783.
- 6) Gregor, P.D., M. Sawadogo and R.G. Roeder (1990). The adenovirus major late transcription factor USF is a member of the helix-loop-helix group of regulatory proteins and binds DNA as a dimer. Genes Dev. **4**:1730-1740.
- 7) Blackwell, T.K., L. Kretzner, E.M. Blackwood, R.N. Eisenman, and H. Weintraub (1990). Sequence-specific DNA binding by the c-Myc protein. Science **250**:1149-1151.
- 8) Bendall, A.S. and P.L. Molloy (1994). Base preference for DNA binding by the bHLH-Zip protein USF: effects of MgCl₂ on specificity and comparison with binding of Myc family members. Nucleic Acids Res. **22**:2801-2810.
- 9) Davis, A.C., M. Wims, G.D. Spotts, S.R. Hann, and A. Bradley. 1993. A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous females. Genes Dev. **7**:671-682.

10) Siritto, M., Q. Lin, J.M. Deng, R.R. Behringer, and M. Sawadogo. 1998. Overlapping roles and asymmetrical cross-regulation of the USF proteins in mice. Proc. Natl. Acad. Sci. USA **95**:3758-3763.

11) Luo, X. and M. Sawadogo (1996). Antiproliferative properties of the USF family of transcription factors. Proc. Natl. Acad. Sci. USA **93**:1208-1313.

12) Siritto, M., Q. Lin, T. Maity and M. Sawadogo (1994). Ubiquitous expression of the 43- and 44-kDa forms of transcription factor USF in mammalian cells. Nucleic Acids Res. **22**:427-433.

13) Schreiber, E., P. Matthias, M. M. Muhler, and W. Schaffner (1989). Rapid detection of octamer binding proteins with mini-extracts prepared from a small number of cells. Nucleic Acids Res. **17**:6419.

14) Luo, X. and M. Sawadogo (1996). Functional domains of transcription factor USF2: atypical nuclear localization signals and context-dependent transcriptional activation domains. Mol. Cell. Biol. **16**:1367-1367.

15) Qyang, Y., T. Lu, P.M. Ismail, D. Krylov, C. Vinson and M. Sawadogo. Cell type-dependent activity of the ubiquitous transcription factor USF in cellular proliferation and transcriptional activation (submitted for publication).

16) Krylov, D., D. Echlin, K. Kasai, H. Arnheiter, E. Taparowsky, and C. Vinson. 1997. A general method to design dominant negative to bHLHZip proteins that abolish DNA binding. Proc. Natl. Acad. Sci. USA **94**:12274-12279.

PUBLICATIONS

Articles:

Financial support from the Department of the Army will be acknowledged in at least two publications:

- 1) Qyang, Y., T. Lu, P.M. Ismail, D. Krylov, C. Vinson and M. Sawadogo. Cell type-dependent activity of the ubiquitous transcription factor USF in cellular proliferation and transcriptional activation (a revised version of this manuscript is currently under review for publication in Mol. Cell. Biol.).
- 2) Ismail, P.M., T. Lu, and M. Sawadogo. Partial or complete loss of USF transcriptional activity in breast cancer cell lines. (manuscript currently in preparation).

Abstracts:

- 1) Sawadogo, M., Ismail, P. and Lu, T. Activity of USF in breast cancer cell lines. Department of Defense Breast Cancer Research Program Meeting Era of Hope, Washington, DC, November 1997.

Personnel who received pay from this grant:

- 1) Preeti M. Ismail, Ph.D. (postdoctoral fellow, 100% effort on this project)
- 2) Tao Lu, Ph.D. (postdoctoral fellow, 20% effort on this project)
- 3) Yibing Qyang, M.S. (graduate student, 5% effort on this project)
- 4) Hui-Xin Yang, M.S. (Research Assistant I, 5% effort on this project)